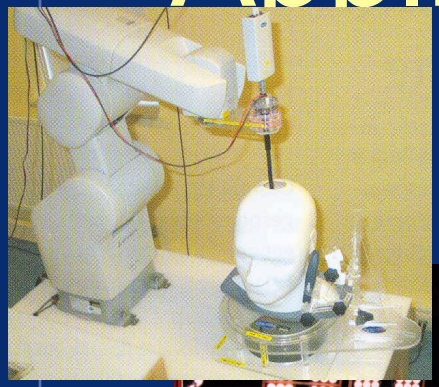
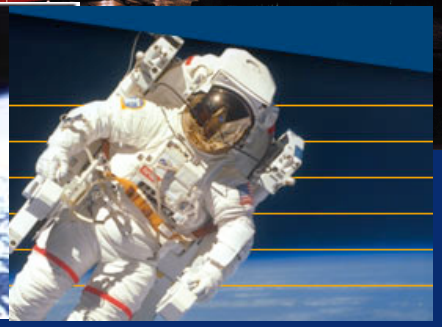
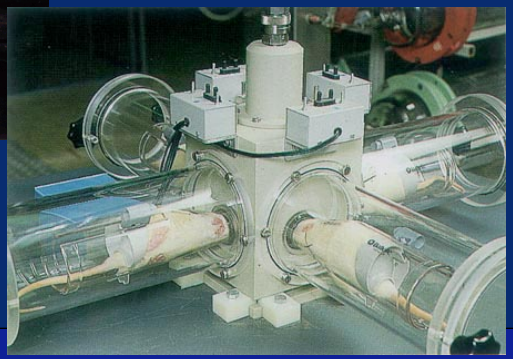
Netherlands Organisation for Applied Scientific Research







- 70 years of experience
- Annual turnover >500 million euro
- Independent organisation
- 14 Research Institutes
- 5000 employees

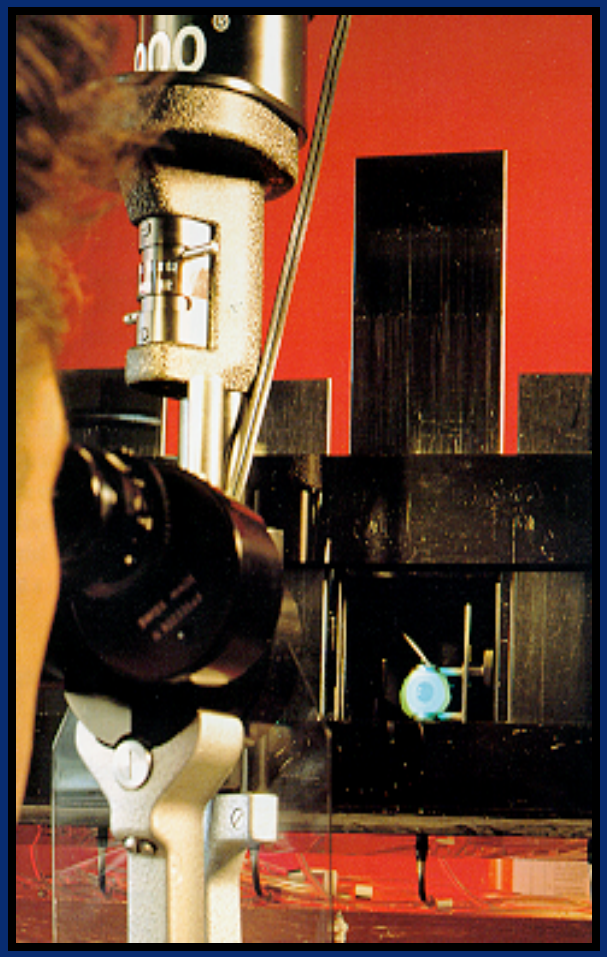
Menk Prinsen

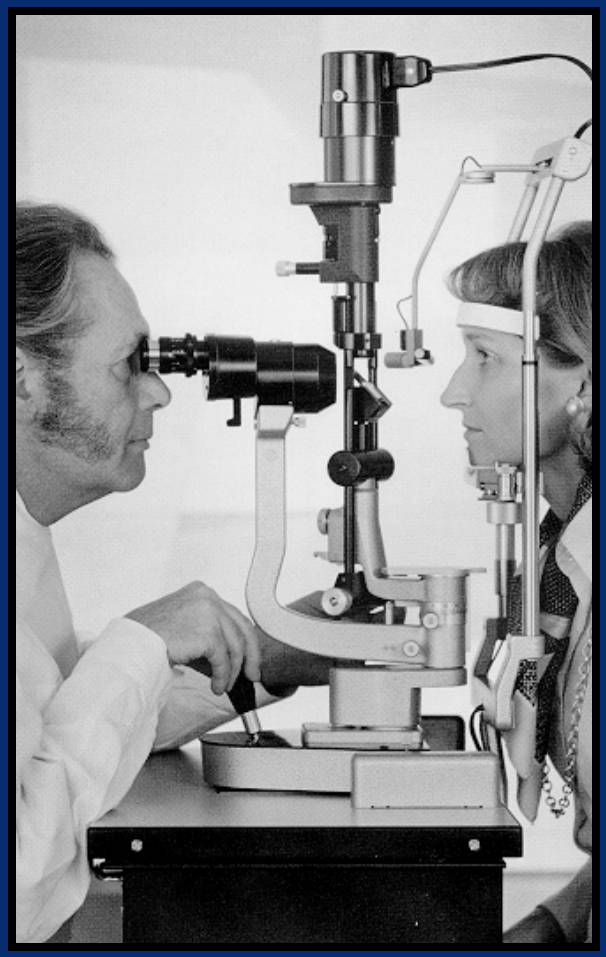
- Toxicologist
- Responsible for acute toxicology since 1981
- Practical experience with IRE, ICE, BCOP and HET-CAM

ICE in protocol toxicology

- **Developed by Herman Koëter and Menk Prinsen**
- **Based on method of Burton (1981)**
  - Isolated rabbit eyes in superfusion apparatus
  - 10-sec. application
  - slit-lamp examination cornea (swelling, opacity, fluorescein)
- **Embedded in acute toxicology testing since 1992**
  - Eye irritation
  - Skin irritation
  - acute oral toxicity
  - Acute dermal toxicity
  - sensitization

ICE in protocol toxicology





ICE – Test design

Slaughterhouse species

- **Bovine**

  eyes too big

  cornea too thick (mild irritants)

- **Porcin**

  difficult to collect

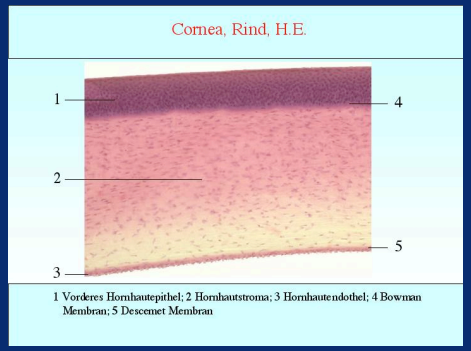
  cornea too thick (mild irritants)

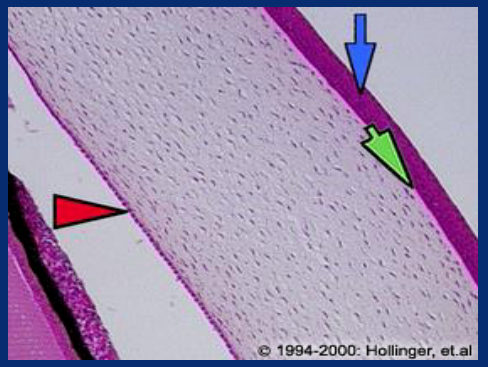
- **Chicken**

  easy to collect

  cornea comparable to rabbit/man

  very uniform

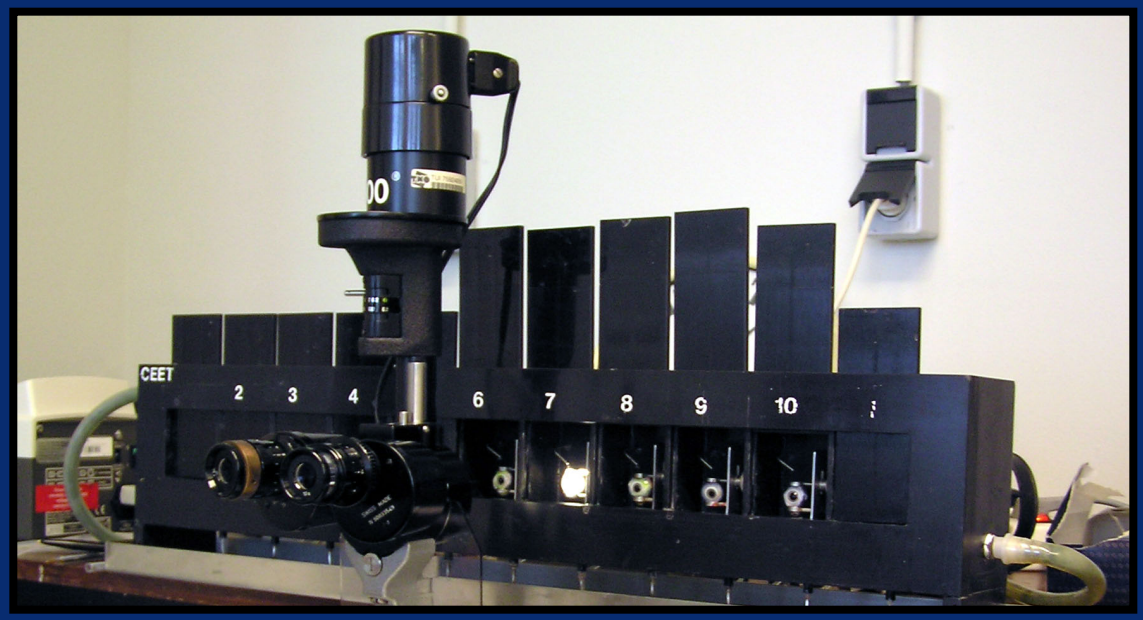




ICE– Test design

- Ross spring chicken
- 50.000 per day
- Sedation by electroshock
- Collection during bleeding
- Uniform and robust

ICE – Test design



Superfusion apparatus
- 11 chambers with sliding doors
- Water mantle
- Saline inlet and outlet

ICE – Test design

Waterbath/pump
- Control set at 37 ^{o}C

ICE – Test design

Peristaltic pump
- 12 channel
- Passes water mantle
- Saline drip

ICE – Test design

• Haag-Streit Slit lamp microscope	9,000 $
• Peristaltic pump (12-channel)	2,500 $
• Water-bath	750 $
• Superfusion apparatus	2,500 $
• Eye clamps	250 $
Total	15,000 $

ICE – Test design

Transport boxes
- 3 heads per box
- Moistened tissue
- Ambient temperature

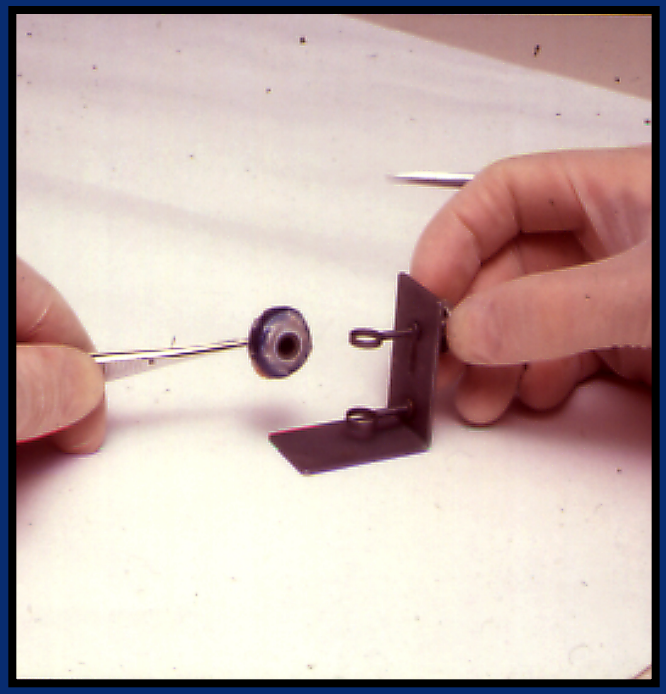
ICE – Test design

- clamps
- Bent scissors
- forceps

ICE – Test design

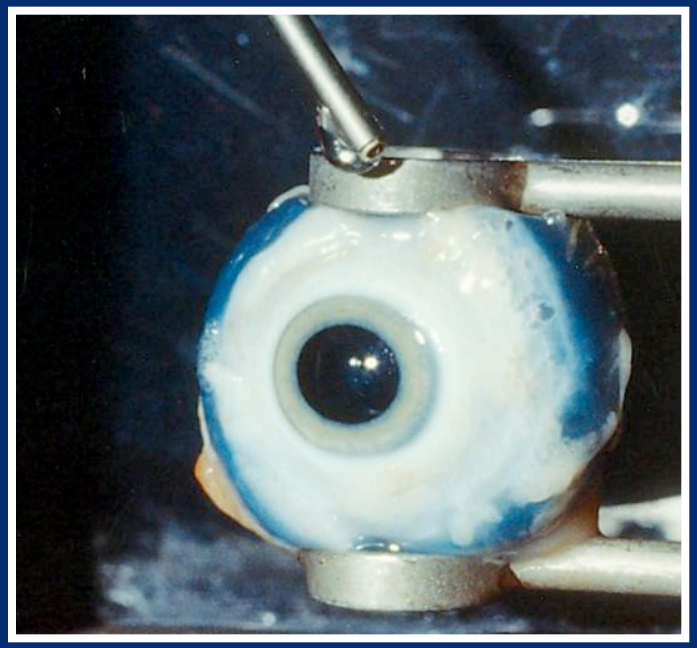
Fluorescein
- Detection of damaged epithelium
- One drop and immediate rinsing

ICE – Test design



- Enucleation within 10 seconds

ICE – Test design

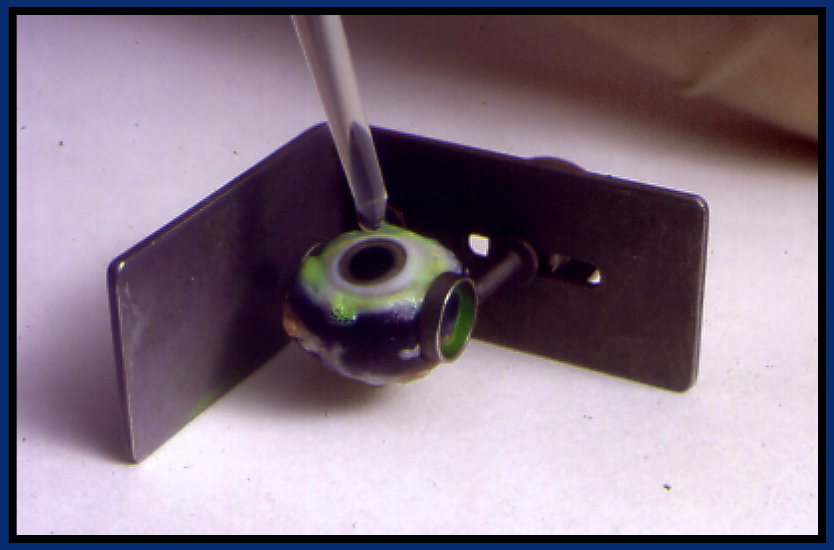


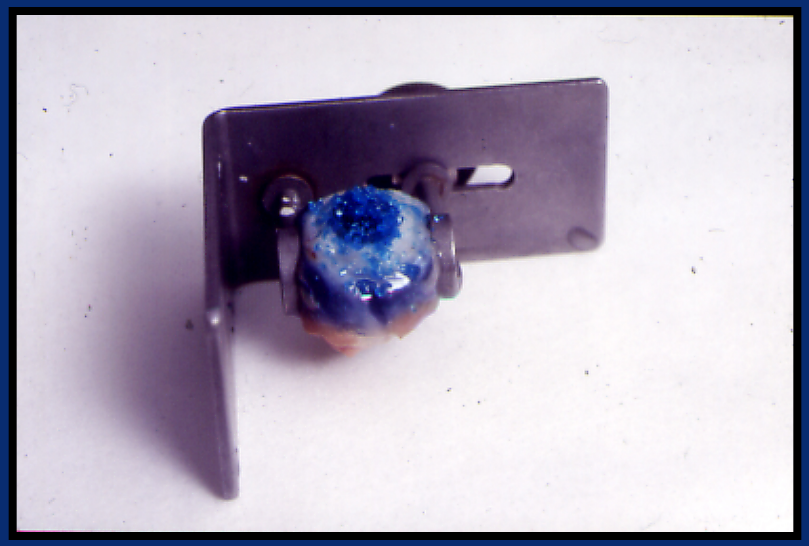
General conditions
- Saline drop every 2-3 seconds
- Room temperature ca 32 °C
- Acclimatization ca 45 min

ICE – Test design

Baseline measurements
- Corneal opacity
- Corneal thickness
- Fluorescein retention

ICE – Test design

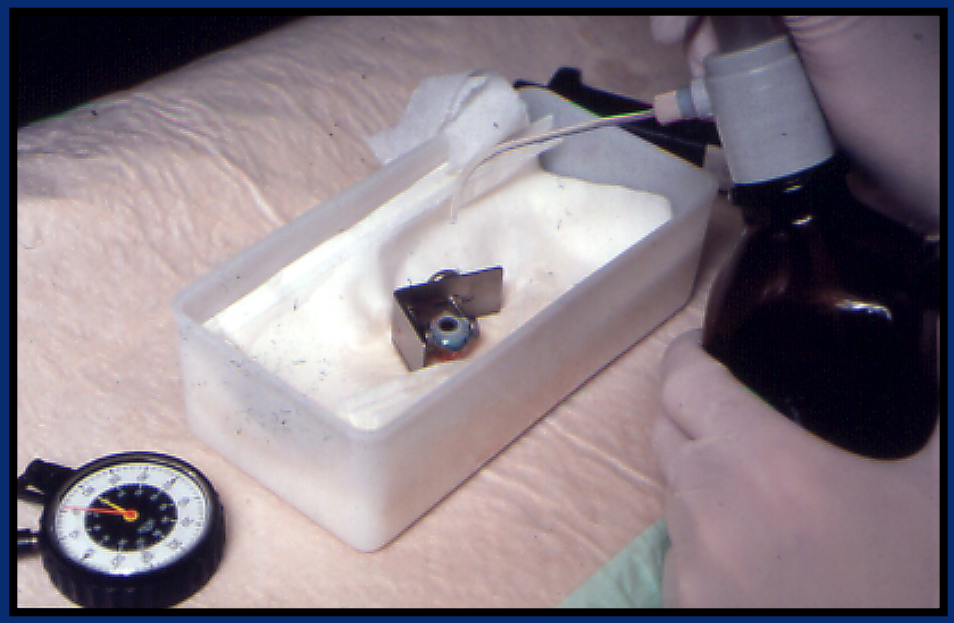




Liquids 30 µl Solids 30 mg

10 second application

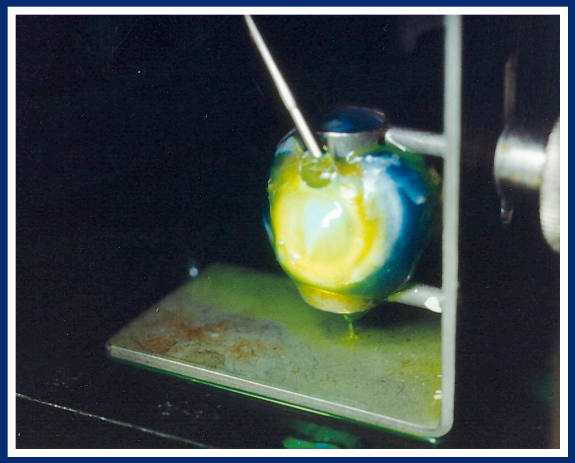
ICE – Test design



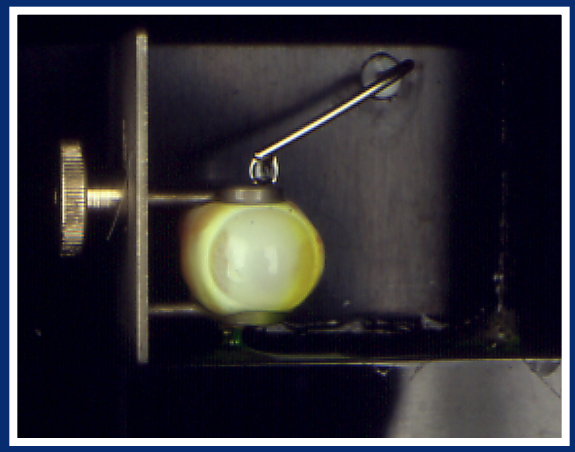
Rinsing with 20 ml of physiological saline (ambient)

ICE – Test design

Chicken eye

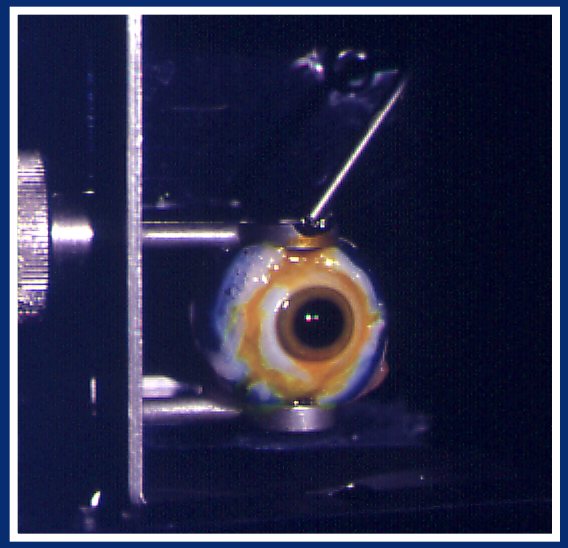


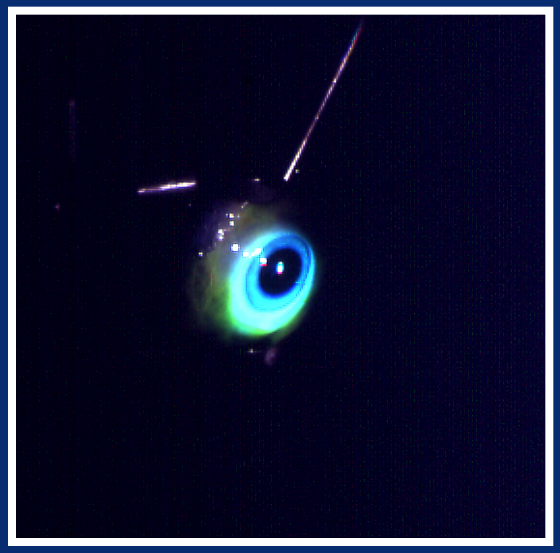
Rabbit eye



Determination of opacity by slit-lamp microscope examination

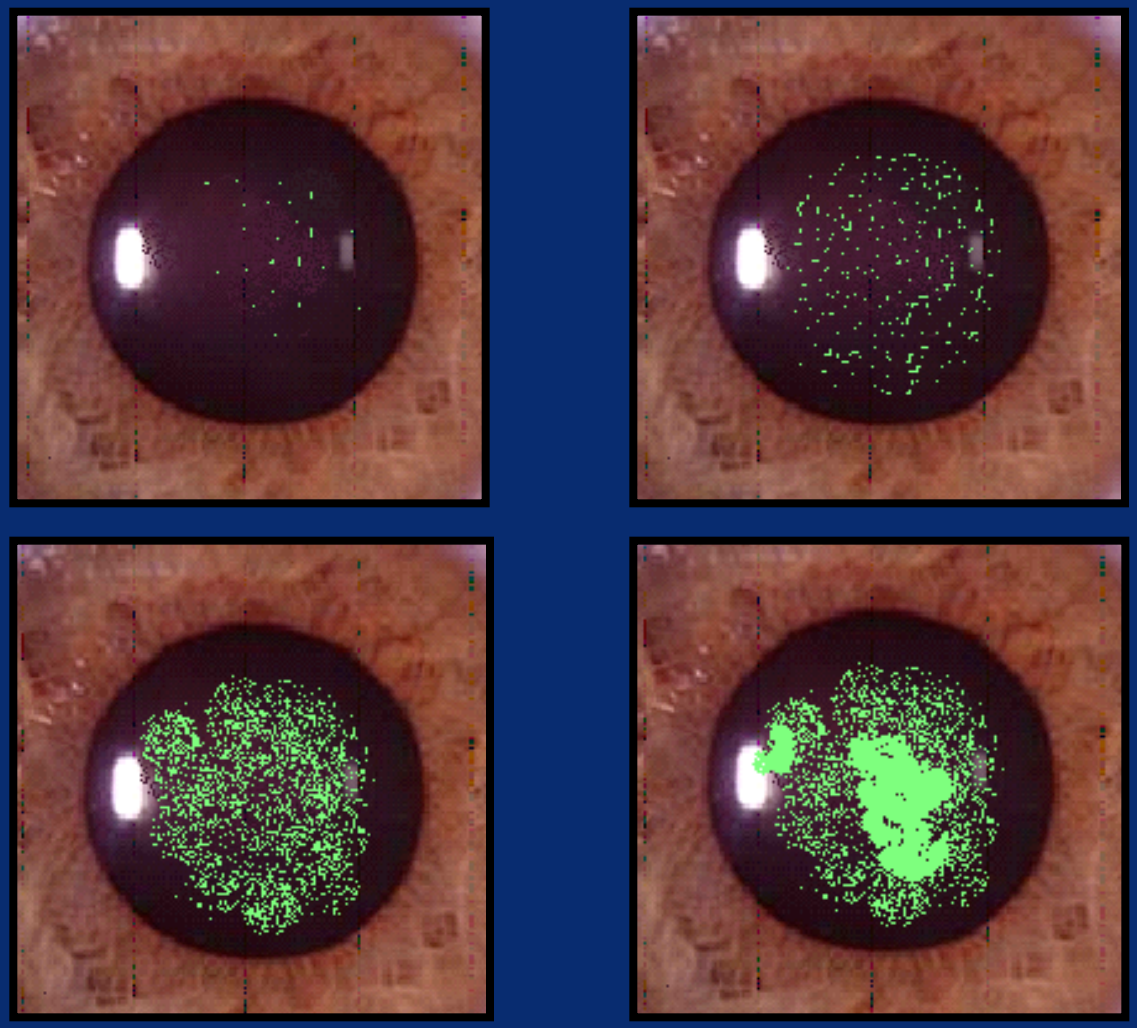
ICE – Test design



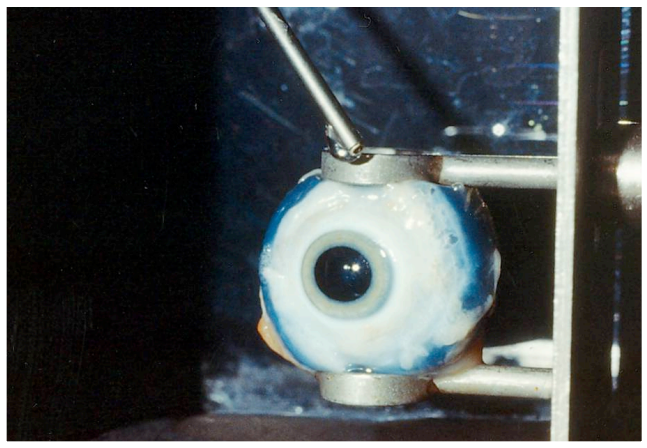


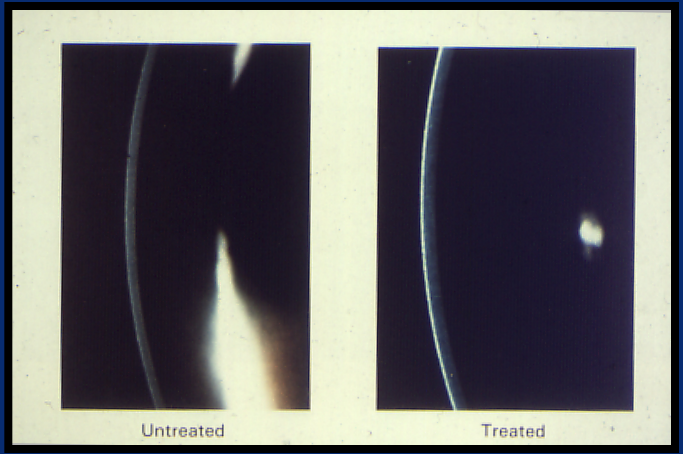
Determination of fluorescein retention by slit-lamp microscope examination

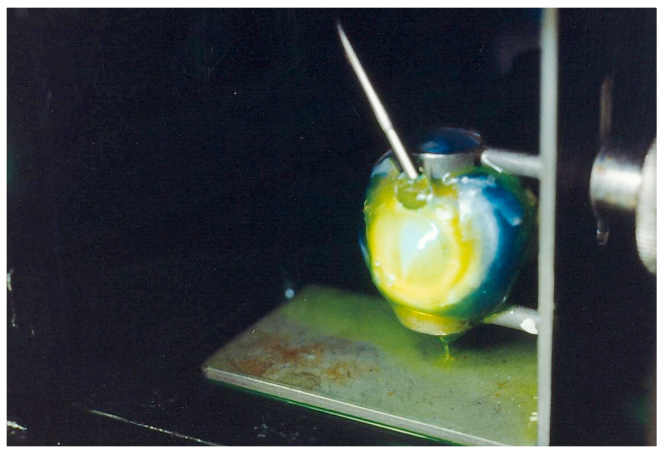
ICE – Test design

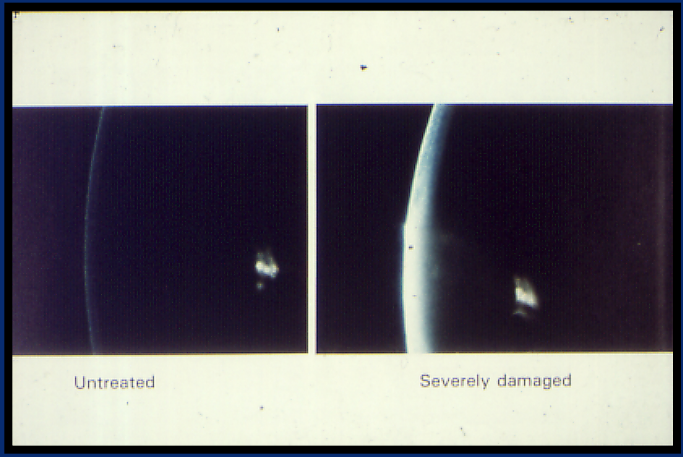


ICE – Test design



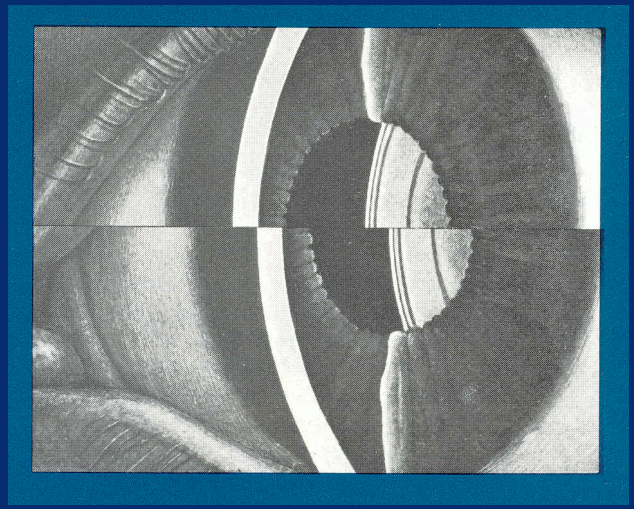






Determination of corneal thickness by slit-lamp microscope examination

ICE - Test design



Determination of corneal thickness by slit lamp microscope examination

ICE - Prediction model

- Corneal swelling 0-60%
- Corneal opacity 0-4
- Fluorescein retention 0-3
- Other

Grading irritancy: none/slight/moderate/severe

(EC)-classification: NI/R36/R41

ICE - Prediction model

Corneal opacity:

mean max. opacity score	Category	
• 0.0 - 0.5	I	(no effect)
• 0.6 - 1.5	II	(slight)
• 1.6 - 2.5	III	(moderate)
• 2.6 - 4.0	IV	(severe)

ICE - Prediction model

Fluorescein retention:

mean score	Category
• 0.0 - 0.5	I
• 0.6 - 1.5	II
• 1.6 - 2.5	III
• 2.6 - 3.0	IV

ICE - Prediction model

Corneal swelling:

Mean corneal swelling (%)		Category
-5 - 5		I
6 - 12		II
13 - 18	(>75 min. after treatment)	II
	(<75 min. after treatment)	III
19 - 26		III
27 - 32	(>75 min. after treatment)	III
	(<75 min. after treatment)	IV
>32		IV

ICE - Prediction model

Classification	Combinations of the three categories
• A. Not irritating	3 x I
	2 x I, 1 x II
• B. Slightly irritating	3 x II
	2 x II, 1 x I
	2 x II, 1 x III
	2 x I, 1 x IV[1]
	1 x I, 1 x II, 1 x III[1]
• C. Moderately irritating	3 x III
	2 x III, 1 x II
	2 x III, 1 x IV2
	2 x III, 1 x I[1]
	2 x II, 1 x IV[1]
	1 x II, 1 x III, 1 x IV[1]
• D. Severely irritating	3 x IV
	2 x IV, 1 x III
	2 x IV, 1 x II[1]
	2 x IV, 1 x I[1]
	immediate corneal opacity score 3
	corneal opacity score 4
	severe loosening of epithelium

[1] Combinations of categories less likely to occur.
[2] The combination of 2 x III, and 1 x IV borderline case between moderately irritating and severely irritating

ICE - Prediction model

Classification	Combinations of the three categories
• NI = not irritating 3 x I	
(combination of A and B)	3 x II
	2 x I, 1 x II
	2 x II, 1 x I
	1 x I, 1 x II, 1 x III[1]
• R36 = irritating 3 x III	
(combination of B and C)	2 x II, 1 x III
	2 x III, 1 x II
	2 x III, 1 x IV
	2 x I, 1 x IV[1]
	2 x II, 1 x IV[1]
	2 x III, 1 x I[1]
	1 x II, 1 x III, 1 x IV[1]
• R41 = severely irritating	3 x IV
	2 x IV, 1 x III
	2 x IV, 1 x II[1]
	2 x IV, 1 x I[1]
	immediate corneal opacity score 3
	corneal opacity score 4
	severe loosening of epithelium

[1] Combinations of categories less likely to occur.

The combination of 3 x II borderline case between non-irritating and irritating

The combination of 2 x III, and 1 x IV borderline case between irritating and severely irritating

ICE – Positive control

TNO-voorschrift
ITV/IRR/006 F1 Versie 1 Pagina 1/1
1 mei 2002

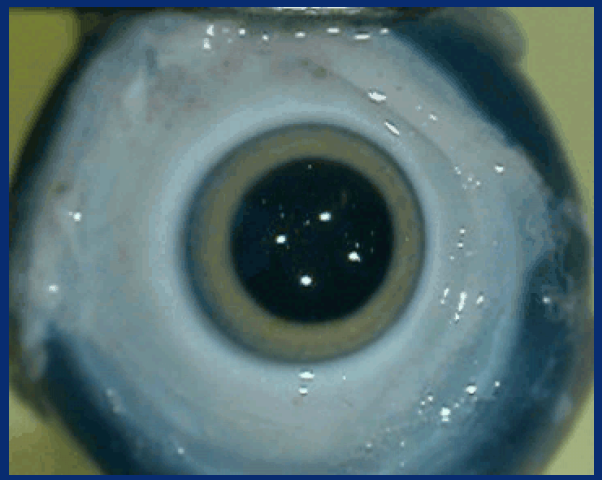
TNO Voeding
Formulier : ITV/IRR/006 F1
Titel : scoreformulier Chicken Enucleated Eye Test (CEET)

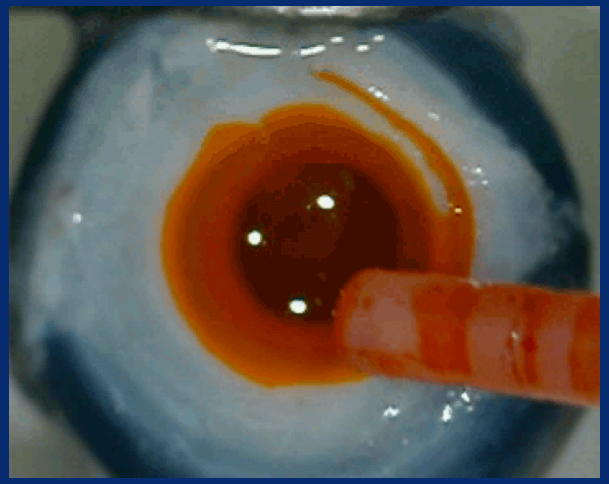
Test compound(s) acetic acid 10% Liquid/solid/viscous/warmed/ground
Project no. ICCVAM Hydrophilic/hydrophobic
Date and signature 8 dec 2004 Appearance clear colourless

Eye no.	Corneal thickness (instrument units) 9.30	10.15	10.45	11.30	12.15	13.15	14.15	Corneal opacity						Other effects	Fluorescein Retention	
T = ☞	-45	0	30	75	120	180	240	0	30	75	120	180	240		0	30
1	60	60	68	72	74	76	76	0	3	3	3	2	2	Immediate opacity 3	0	n.d.
sw%			13	20	23	27	27									
2	63	63	73	77	78	79	79	0	3	3	3	3	3	"	0	n.d.
sw%			16	22	24	25	25									
3	65	65	76	79	81	84	85	0	3	3	3	2	2	"	0	n.d.
sw%			17	22	25	29	31									
4														opacity mainly in epithelium		
sw%																
5	61	60	60	59	59	59	60	0	0	0	0	0	0		0	0
sw%			0	-2	-2	-2	-2									
6																
sw%																
7																
sw%																
8																
sw%																
9																
sw%																
10																
sw%																
11																
sw%																
Initials	mr	mr	mr	mp	mp	mp	mp	mp	mp	mp	mp	mp	mp		mp	mp
Mean 1			15	21	24	27	28		3	3	3	2.3	2.3			n.d.
Mean 2																
Mean 3																

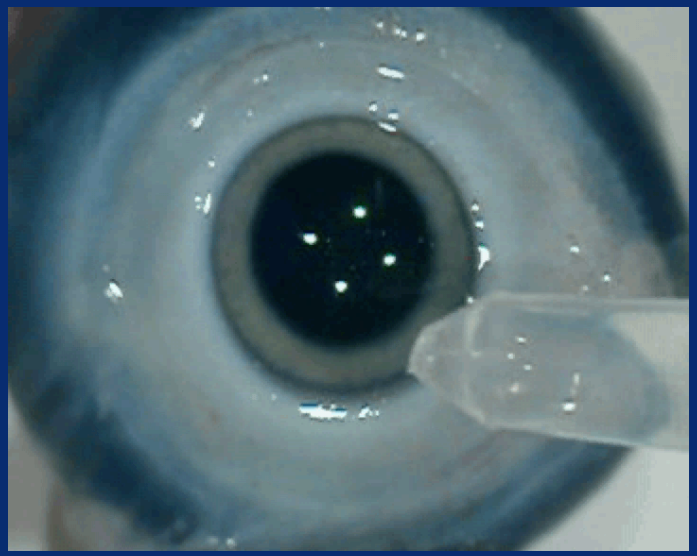
compound 1: Categories III; IV; n.d. Classification R41
compound 2: Categories Classification
compound 3: Categories Classification

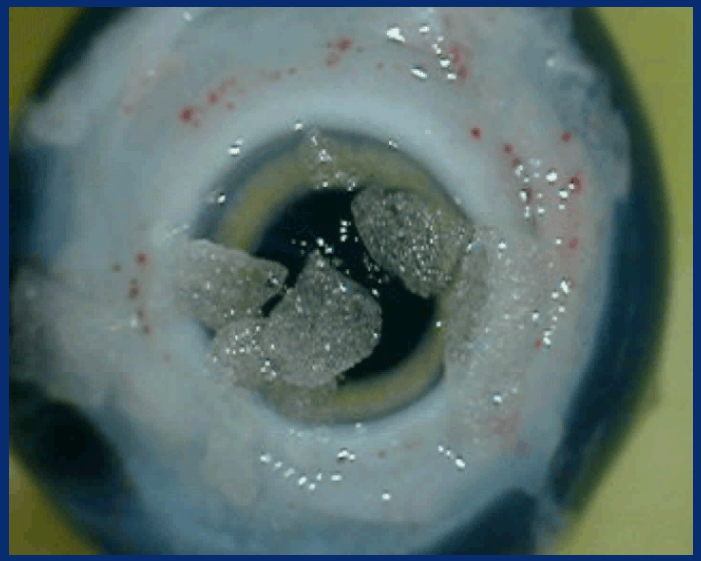
n.d. = not dissectable epithelium fixated





ICE – severe irritancy

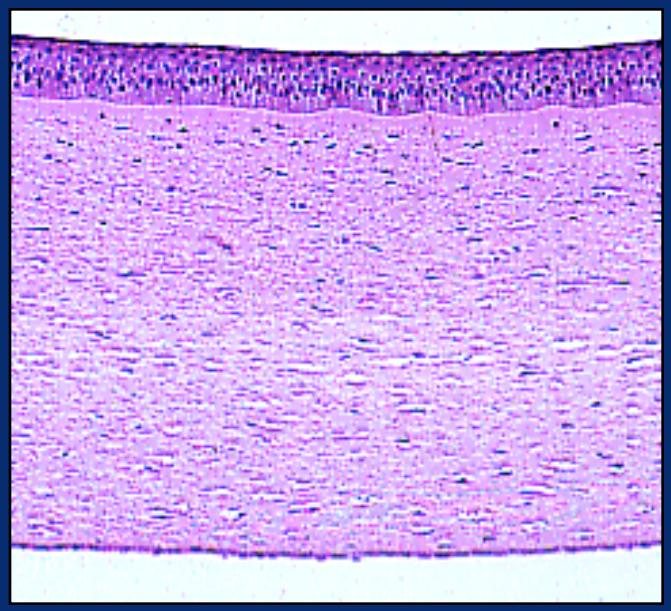


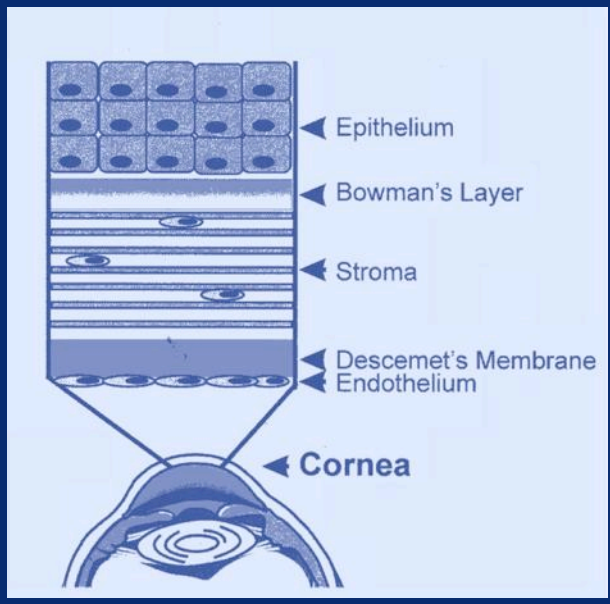


Sodium hydroxide 10% and solid

ICE - Optimization

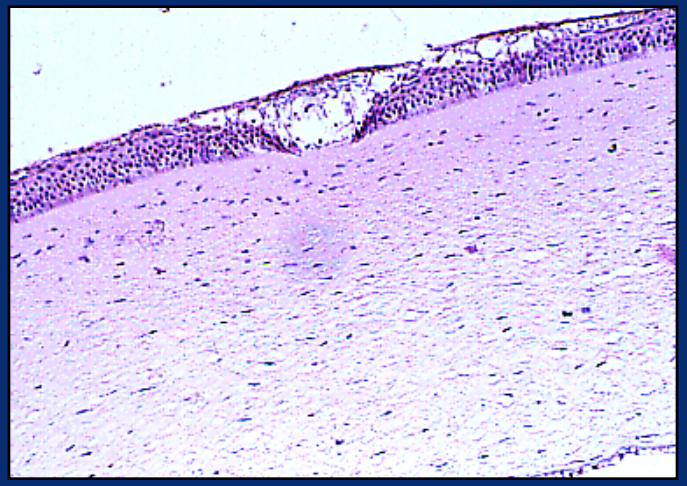
- **Histopathology of the cornea – depth of injury**





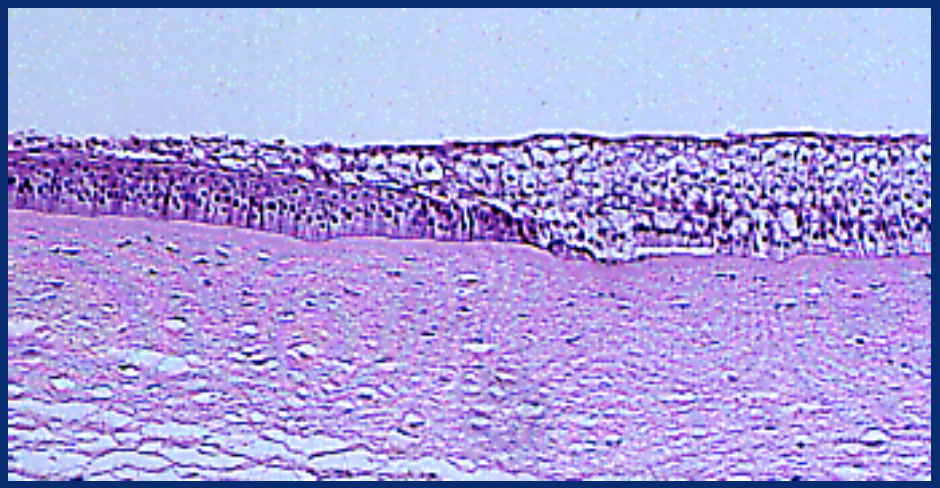
ICE - Optimization

- Histopathology of the cornea – depth of injury



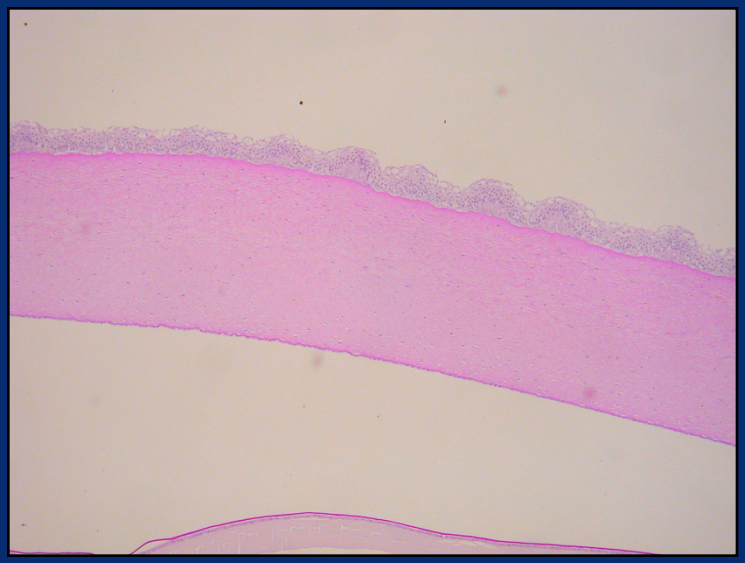
ICE - Optimization

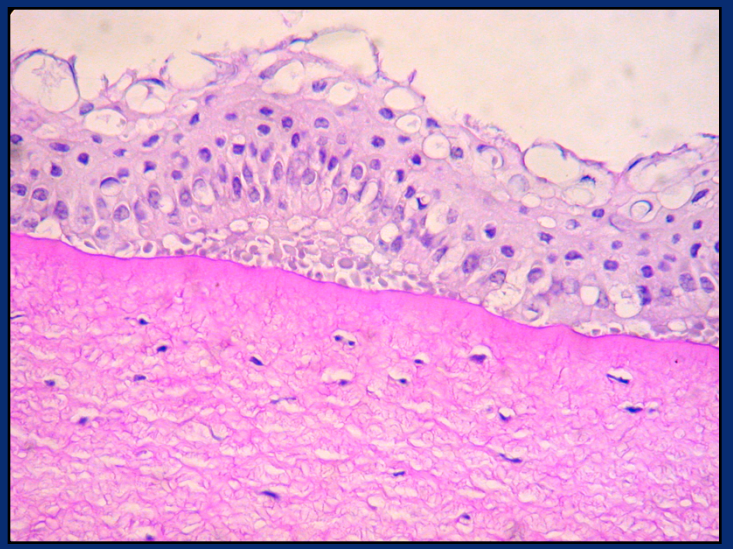
- Histopathology of the cornea – depth of injury



ICE - Optimization

- Histopathology of the cornea – depth of injury





ICE in protocol toxicology

Eye irritation

- **prescreen OECD 405/EC B.5/EPA 870.2400**
  Stripped non-GLP procedure
  Full GLP ICE in case of severe irritancy
- **stand-alone**
  Cosmetic/household (company policy)